Review Article

The tissue factor pathway of coagulation

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ABSTRACT: Coagulation is classically divided into two pathways; the extrinsic and intrinsic. In a revised coagulation pathway, the tissue factor pathway, these two pathways are combined and represented as one. In this scheme, coagulation is initiated by tissue factor, a membrane bound protein, which combines with factor VII or factor VIIa to form a complex at the site of tissue damage. The resulting factor VII/tissue factor or factor VIIa/tissue factor complex then acts not only on factor X, forming factor Xa, but can also activate factor IX to factor IXa. Factor Xa is important both for the continuation of the coagulation cascade, with ultimate thrombin and fibrin formation, as well as playing an important role in inhibition when bound to tissue factor pathway inhibitor. Multiple Kunitz-type domains in the inhibitor allow it to bind to and inactivate both factor Xa and the factor VII(a)/tissue factor complex. Activation of factor XI is therefore necessary to sustain coagulation through its activation of additional factor IX, with subsequent factor Xa production. Conflicting experimental results makes validation of this hypothesis difficult and unresolved issues involving crucial steps of the proposed pathway remain.

Keywords: Coagulation, tissue factor, factor VII, factor XI, tissue factor pathway inhibitor.

Introduction

In 1964 two interpretations of the sequence of events leading to the production of fibrin were published; an enzyme cascade (1) and the waterfall sequence (2). They formed the basis of the intrinsic pathway of coagulation depicting coagulation as a series of step wise reactions initiated by the activation of factor XII to factor XIIa. Additional proenzyme to enzyme trans-formations followed, with each step being activated by the product of the preceding one. In the final step, thrombin converted fibrinogen to fibrin. The extrinsic pathway joined the intrinsic pathway at factor X (1-3) (Figure 1).

It is now known that factors V and VIII are not zymogens, activated to enzymes as coagulation proceeds, but precursors of the cofactors, factor Va and factor VIIIa (4). It is also known that both the intrinsic and extrinsic pathways are affected by a number of feed back mechanisms which serve to complicate the simple sequence of events proposed.

The intrinsic pathway was considered essential because the factors deficient in haemophilia, factors VIII and IX, were components of this pathway. The extrinsic pathway was allocated a position of secondary importance.

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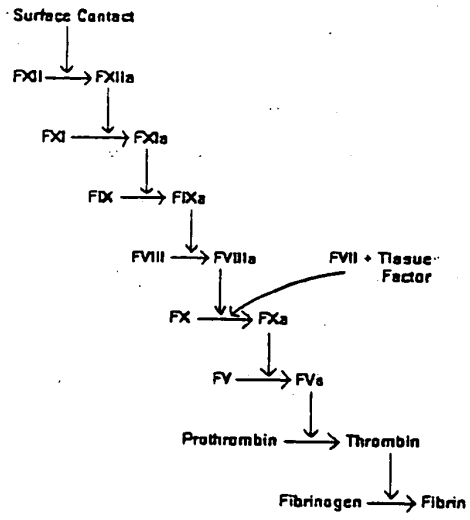

Figure 1. The cascade or waterfall hypothesis of coagulation as proposed in 1964. Clotting was initiated when blood came in contact with a foreign surface leading to the activation of factor XII. The activation of factor X by factor VIIa and tissue factor is also shown. The role of calcium and phospholipid was uncertain at this time.

It is well documented, however, that patients who are deficient in one of the contact factors, involved in the initiation of the intrinsic pathway, do not have an abnormal bleeding tendency although their partial thromboplastin time is extended (5,6). In comparison,

factor VII deficient patients present with variable histories of bleeding and bruising and severely affected paticnts can have clinical manifestations analogous t those seen in haemophilia A and B (7,8). Evidence also exists that the factor VIIa/tissue factor complex (FVIIa/TF) can activate factor IX as well as factor X (9). These facts, in conjuncti n with the rediscovery of the tissue factor pathway inhibitor (TFPI) originally described by Hjort in 1957 (10), has led to the formulation of a revised hypothesis for coagulation, namely, the tissue factor pathway (11-13).

Overview of the tissue factor pathway

The tissue factor pathway combines the two classical pathways, the extrinsic pathway and the intrinsic pathway and represents them as one (Figure 2). Coagulation is initiated when tissue factor, released at the site of injury, forms a complex with factor VII or factor VIIa. This complex then activates factor X in the presence of calcium ions to factor Xa, and factor IX to factor IXa (14). The factor Xa produced can participate in a number of different reactions:

1. Activation of factor VII/tissue factor complex (FVII/TF) to FVIIa/TF (15,16);
2. conversion of prothrombin to thrombin by prothrombinase, a complex consisting of factor Xa, factor Va, calcium ions and phospholipid (4,17);
3. activation of the cofactors, factor V and factor VIII (4), and
4. formation of a complex with TFPI to inactivate FVIIa/TF (18).

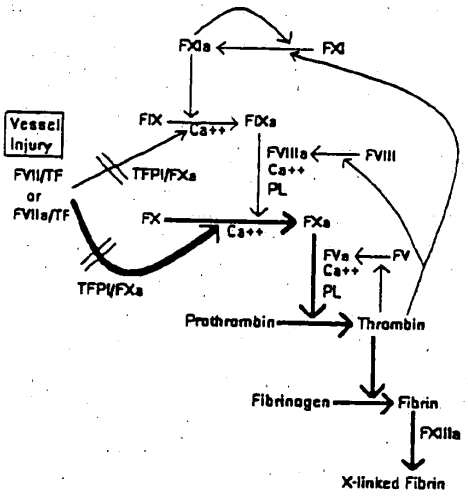

Figure 2. The tissue factor pathway. Initiation of coagulation occurs following vessel injury and the formation of factor VII/tissue factor complex (FVII/TF) or factor VIIa/tissue factor complex (FVIIa/TF). This triggers the extrinsic pathway, highlighted by heavy arrows.

The formation of the quarternary complex between factor Xa, TFPI and FVIIa/TF also results in the inactivation of factor Xa (18) before sufficient fibrin can be formed. To enable coagulation to proceed, additional factor Xa production is crucial and this is generated via factor XIa. The factor XIa does not result from contact activation as in the early stages of the classical intrinsic pathway, but forms when trace amounts of thrombin, produced in initial prothrombinase reactions, cleave factor XI. This production of factor XIa is considered essential for sustaining coagulation.

Factor XIa acts on factor XI in an autocatalytic manner and also converts factor IX to factor IXa in the presence of calcium ions. Factor IXa then activates more factor X to factor Xa with factor VIIIa the cofactor in this reaction. The factor VIIIa results from the activation of factor VIII by factor Xa or thrombin (4).

Coagulation proceeds with activated factor V, factor Xa, calcium ions and phospholipid forming the prothrombinase complex which cleaves prothrombin to thrombin. Thrombin then releases fibrinopeptide A and B from fibrinogen leaving fibrin monomer, which polymerises and then is stabilised by factor XIIIa (14).

Initiation of the tissue factor pathway

The formation of a complex between factor VII and tissue factor or factor VIIa and tissue factor is recognised as the first step in the tissue factor pathway of coagulation. This is contrary to previous beliefs that coagulation was dependent on activation of the intrinsic pathway, not the extrinsic, a concept supported by the inability of the prothrombin time to detect any abnormality in haemophiliac plasma. The prothrombin time reagent incorporates high concentrations of thromboplastin to achieve clotting times, with normal plasmas, of around 12 seconds. If dilute thromboplastin is used, haemophiliacs do record clotting times longer than normal control plasmas. This fact was documented as early as 1951 by Biggs and MacFarlane (19). They found that diluting a thromboplastin reagent to give normal plasma a clotting time of 40 seconds, or greater, resulted in an abnormal clotting time with haemophiliac plasma. Similarly, in 1982, Marlar *et al.* (20), studied the activation of factor X, in various factor deficient plasmas, using a range of thromboplastin dilutions. At the 1/24 dilution of thromboplastin, there was a 50% decrease in factor X activation using factor VIII and factor IX deficient plasmas compared with normal and factor XI deficient plasmas. Activation fell to 10% of normal when the thromboplastin was diluted to 1/260.

This *in vitro* discovery can be explained within the context of the tissue factor pathway. The amount of factor Xa produced in plasma is dependent on the activation of factor X either directly by FVIIa/TF or indirectly by FVIIa/TF, via factor IXa. Subsequent activation of prothrombin then relies on the formation of the prothrombinase complex which incorporates factor Xa and factor Va in a 1:1 stoichiometry. Factor Va is the limiting factor, having a concentration in plasma much lower than that of factor Xa. When the concentration of thromboplastin is high, sufficient factor X is activated by the FVIIa/TF to saturate all the available factor Va. Factor Xa produced via factor IXa will be in excess. As a result, a reduced contribution to factor Xa production via factor IXa, as in haemophiliac plasma, will not be reflected in the prothrombin time result. Conversely, when the thromboplastin concentration is much lower, significant amounts of factor X are activated via factor IXa. In this case, low factor VIIIa or factor IXa levels will extend the prothrombin time beyond normal (21).

Factor VII

Factor VII is a single chain, vitamin K dependent protein, with a molecular weight of 50,000 (22). Activation involves the cleavage of a single peptide bond t produce a two chain molecule from the single chain zymogen (23). Both factor VII and factor VIIa have an equal affinity for tissue factor with which they f rm a complex in a calcium dependent reaction. It remains unclear, however, if factor VII/tissue factor complex (FVII/TF) in the presence of calcium ions can activate factors IX and X, or whether FVIIa/TF is required. This has proven difficult to investigate because once the substrate zymogens are activated, the enzymes formed can feedback and activate FVII/TF to FVIIa/TF (24,25).

In 1982, Zur *et al.* (26), in experiments using bovine factor VII, reported that the zymogen contained between one and two percent the activity of factor VIIa and it was concluded that factor VII, in complex with tissue factor, possessed sufficient activity to initiate coagulation. This was supported more recently in an experiment referred to by Nemerson (13) in which D-phe L-phe arg chloromethylketone was used to rapidly inactivate factor VIIa in a series of prothrombin time tests. Treated plasma clotted in 16 seconds compared to the 12 second clotting time of normal, untreated plasma, suggesting that FVIIa is not essential for clot formation.

No enzymatic contribution to coagulation by FVII/TF could be demonstrated in studies which prevented the activation of factor VII to factor VIIa in feedback reactions. It was shown that physiologically significant amounts of factor Xa could only be generated by FVIIa/TF and not by FVII/TF when heparin and antithrombin III were included to prevent factor Xa activating factor VII (16). Similarly, human factor VII was unable to activate a variant factor IX which could not activate factor VII after activation (27). Further evidence was obtained when a mutant recombinant factor VII was developed in which Arg152, required for the activation of factor VII by factor Xa, was replaced by a Glu residue (28). The mutant factor VII had minimal enzymatic activity when compared with that of the mutant factor VIIa supporting the proposal that FVII/TF does not activate factor X or factor IX. Doubts concerning conclusions drawn from this experiment were raised by Nemerson (13) who queried whether the activity of the mutant zymogen and native human factor VII should be considered equivalent.

The question then arises that if factor VII zymogen does not have sufficient activity to activate factor X, so that factor VII can itself be activated, how is the initial factor VIIa formed?

Currently, no reactions have been substantiated that explain this, but a number of hypothetical models have been proposed. Firstly, evidence exists that normal plasma contains factor IX activation peptide (FIXP), the peptide that is liberated when factor IXa is generated (29). The level of FIXP in factor XI deficient patients does not vary significantly from normal, but is markedly reduced in factor VII deficient patients. The majority of FIXP present normally in the circulation can therefore be said to come from the activation of factor IX via factor VII and not from activation of the contact factors (29). This supports a hypothesis that factor VIIa/TF complexes are present normally and do not need generating at the onset of clot formation. Other investigators support the presence of low levels of factor VIIa in circulating blood (30,31). Osterud (1990) proposes that circulating enzymes, factors IXa, XIIa or Xa plus ph spholipid are responsible for the activation of FVII/TF to FVIIa/TF (32). Subsequent activation of factor X t factor Xa occurs which can then act as a component of the prothrombinase complex to convert prothrombin to thrombin or activate more FVII/TF to FVIIa/TF.

Another possibility is that factor Xa is formed in a FVIIa/TF independent reaction. Factor X can bind to the integrin, Mac-1 on ADP activated monocytes, and be cleaved to an active protease with functional factor Xa coagulant activity. It has been shown that physiologically significant levels of ADP can be present during haemostasis (33). Reports also exist which support the autoactivation of factor VII by factor VIIa in the presence of a positively charged surface (34) and tissue factor (35). Rapaport and Rao (1992) (36), however, found the autoactivation to proceed slowly, unaffected by the addition of recombinant factor VIIa, raising doubts about the reaction's physiological significance.

Tissue factor

Irrespective of whether or not factor VII has some intrinsic catalytic activity when bound to tissue factor, its cleavage to factor VIIa is accelerated by trace amounts of factor Xa (37). As a result, factor VII which comes in contact with tissue factor (FVII/TF) at the site of injury will be rapidly converted to FVIIa/TF. It has also been shown that factor VIIa in complex with tissue factor, is over one hundred times more efficient as an enzyme than factor VIIa alone (38,39).

Tissue factor, therefore, plays a key role in the initiation of coagulation in the tissue factor pathway. It must be present in a protein/phospholipid complex to express functional activity. Tissue factor apo-protein is not functional in haemostasis (36). It is a membrane bound glycoprotein with three distinct domains: extracellular, hydrophobic and cytoplasmic (40), and has been purified from both bovine and human tissue (41,42). Previously known as coagulation factor III or tissue thromboplastin, the tissue factor gene has been localized to the short arm of chromosome 1 (1pter-> 1p21), but its pattern of inheritance is unknown (43).

There are three membrane-bound, enzyme/cofactor complexes involved in normal coagulation of which FVIIa/TF is one: tenase and prothrombinase being the other two (17). FVIIa/TF is unique because its cofactor, tissue factor, does not require activation to function (41), unlike the cofactors in the other two complexes, factor V and factor VIII, which need to be converted to their active forms (4). As a result, although tissue factor has been demonstrated in many tissues and cells by immunohistochemical staining using monoclonal antibodies to tissue factor apo-protein (36,44), it is not found in direct contact with the blood. Endothelial cells, smooth muscle cells and fibroblasts have all been shown to contain tissue factor, but disruption f the cells is required for expression of its coagulant activity (45).

Tissue factor pathway inhibitor

Coagulation is regulated by a number of natural anticoagulant mechanisms. The action of antithrombin III in neutralizing factors Xa, IXa, XIa and XIIa as well as thrombin is well documented (46–48), as is inhibition of the cofactors, factor Va and factor VIIIa, via the protein C pathway (4,46,49).

There are no physiologically significant inhibitors of either factor VIIa or tissue factor. Normally VIIa/TF complexes are prevented from forming by the lack of exposure of factor VIIa to the tissue factor. Once FVIIa/TF is formed, however, the complex can be inhibited by TFPI in association with factor Xa (50).

This inhibitor has previously been called anticonvertin (10), extrinsic pathway inhibitor (EPI) (50) and the lipoprotein-associated coagulation inhibitor (LACI) (18). TFPI is the name the International Society on Thrombosis and Haemostasis (Amsterdam, The Netherlands), agreed to use in 1991 (51).

There are three intravascular pools of TFPI (52). It is present in plasma bound to lipoproteins, on the endothelium of the microvasculature and less than 2.5%, of the total intravascular pool, is located in platelets. TFPI has not been found on the endothelium of larger vessels or in hepatocytes (53). The endothelium bound TFPI differs structurally from lipoprotein TFPI (54) and can be released into the plasma by intravenous heparin (54–56) causing a marked increase in the plasma level (52–56). Heparin may therefore effect the activity of factor VII and the extrinsic pathway of coagulation, as well as the intrinsic, an outcome which is not monitored in the laboratory with either the APTT or thrombin clotting time (57).

Plasma levels of the inhibitor have been determined by various methods and method summaries have been included in a number of reviews (52,58). The concentration of TFPI has been found to show no variation, in healthy controls, during the day or from month to month (58,59), and levels were not decreased in patients with liver disease or on warfarin therapy in a study using a competitive fluorescent immunoassay to measure plasma concentrations (56). No liver involvement in the production of TFPI has been found. Following elective surgical procedures that caused the patient's fibrinogen to increase, TFPI levels were found to decrease, comparable to the albumin levels. This was probably due to haemodilution and redistribution and indicates that TFPI is not an acute phase reactant (59,60).

Increased levels of the inhibitor have been documented in association with advanced cancer (61), acute ischaemic heart disease (62) and the last trimester of pregnancy (59). As heart disease and pregnancy have also been associated with elevated factor VII levels, a link between factor VII and TFPI levels has been suggested (62). This hypothesis, however, is not supported in a recent study involving the lowering of serum cholesterol in patients with familial hypercholesterolemia (63). The mean decrease in cholesterol was 39%, low density lipoprotein, 46%, and apolipoprotein B, 36%. The level of TFPI fell significantly from a median of 153% to 111%. TFPI activity could not b correlated with either factor VII activity or fact r VII antigen levels (63).

TFPI is a Kunitz-type serine protease inhibitor with three tandemly linked inhibitory domains (64). Two reaction sequences have been shown experimentally to cause the inhibition of FVIIa/TF by TFPI (65,66). The first more widely accepted hypothesis involves two steps (65). Initially the TFPI binds to factor Xa in a reaction, independent of calcium ions, forming TFPI/FXa. The second Kunitz domain is utilised in this step (60) which results in the neutralisation of the factor Xa. In the second step, this complex binds to FVIIa/TF via the TFPI's first Kunitz domain to form the quaternary complex FVIIa/TF:TFPI/FXa. This step is calcium dependent. The role of the third Kunitz domain is unknown.

Subsequently, it was shown in experiments using various combinations of factor VIIa, TFPI and factor X or factor Xa in a continuous flow capillary reactor lined with phospholipid, that factor Xa can associate with FVIIa/TF before complexing with TFPI (66). TFPI is definitely required for the final inhibition of the FVIIa/TF and factor Xa, but unlike the first sequence of reactions TFPI/FXa is not formed at the outset.

Factor Xa is, therefore, made unavailable for the continuation of the coagulation pathway in reaction sequences that involve it in inhibiting the complex that initially activated it. Put another way, FVIIa/TF cannot be inhibited until it cleaves factor X to factor Xa. This negation of factor Xa's activity is used to explain the continued necessity for factors of the intrinsic pathway and their subsequent integration into the tissue factor pathway. It is hypothesised that sufficient factor Xa for sustaining coagulation can only be generated via factor XIa.

The role of factor XI

Evidence supporting a link between the extrinsic pathway and the intrinsic pathway has led to renewed interest in the role of factor XI in sustaining haemostasis following the inactivation of factor Xa in the presence of TFPI and FVIIa/TF. In the revised pathway of coagulation, the function of FVIIa/TF is to initiate coagulation and produce some factor Xa and factor IXa which will lead to minimal thrombin formation before it is rapidly inactivated by FXa and TFPI. The continuation of the revised pathway then relies on the activation of factor XI by trace amounts of thrombin, and subsequent activation of factor IX by the factor XIa. Factor VIII is also activated by the thrombin formed, thereby ensuring that the cofactor, factor VIIIa, will be available for activation of additional factor X by factor IXa and the subsequent production of more thrombin and ultimately fibrin. A number of recent articles review the position of factor XI in the revised pathway of coagulation (67–69).

It is known that the additional generation of factor Xa via the intrinsic pathway is essential because of the severe bleeding suffered by haemophiliacs with either factor VIII or factor IX deficiency (4). Patients deficient in factor XI may also show abnormal bleeding patterns (67,70), supporting a key role for factor XI in coagulation. The correlation between the factor XI level and bleeding tendency is poor, with a stronger association, in bleeding tendency, occurring within families (67). The frequency f the genes responsible for the deficiency is high in Ashkenazi Jews with an

estimated 5.5% to 11% of the total population heterozygous (71). It has been shown that two different point mutations are responsible for about 96% of the factor XI deficiencies among the Ashkenazi Jews (72).

The activation of factor XI by factor XIIa in the initial steps of contact activation in the intrinsic pathway is well kn wn (73) (Figure 1). In 1991, two separate reports (74,75) showed that factor XI could be activated by thrombin, in purified systems, to produce an identical factor XIa to that produced by the action of factor XIIa, and capable of activating factor IX in the presence of calcium ions. The efficiency of both thrombin and factor XII to activate factor XI in the absence of cofactors was poor, although it was deduced that thrombin would be more potent in plasma (74). In the study conducted by Gailani and Broze (1991) (74), the activation of factor XI by thrombin was found to be increased 2000 fold by dextran sulphate. Activation by dextran sulphate also occurred when thrombin was removed from the test system. This supports the autoactivation of factor XI to factor XIa. It suggests that some of the initial effect of dextran sulphate, on the ability of thrombin to activate factor XI, was in fact due to autoactivation of the factor XI, probably initiated by trace amounts of factor XIa. The autoactivation of factor XI to factor XIa was supported by Naito and Fujikawa (1991) (75) who also tested the effect of other negatively charged materials, but only dextran sulphate resulted in autoactivation. They did find that heparin and sulphatide also activated factor XI in the presence of thrombin, but with greatly reduced efficiency.

In 1993, Gailani and Broze (76) continued their investigations in plasma systems and reconfirmed that factor XI could be activated in the absence of factor XII. They did admit, however, that the concentration of sulphatides used in the experiments was unlikely to be physiological and the search for the *in vivo* cofactor should continue.

The above conclusions supporting the physiological relevance of the activation of factor XI by thrombin are disputed by some authors. (77,78). Fibrinogen has been found to block both the autoactivation of factor XI and its activation by thrombin, but not to effect activation by factor XIIa (77). In this study, however, the polymerization of fibrin was blocked thus removing a normal *in vivo* product which may be a requirement for thrombin to activate factor XI (76). Brunnee *et al.* (1993) (77), in experiments designed to study factor XI's activation in plasma, also concluded that factor XII and a suitable surface were mandatory for factor XIa production.

Discussion

In 1977, Radcliffe *et al.* (79), showed that fragments from the activation of factor XII could activate factor VII. It was suggested that the intrinsic pathway and extrinsic pathway were linked in the early stages of clot formation, with initiation of the intrinsic pathway priming the extrinsic pathway.

In the tissue factor pathway of coagulation this hypothesis is reversed, with the extrinsic pathway initiating coagulation and the intrinsic pathway sustaining it. Coagulation is triggered at the site f injury when tissue factor complexes with factor VII or factor VIIa in plasma and activates factors IX and X to factors IXa and Xa respectively. Fact r Xa then binds to TFPI to inhibit FVIIa/TF. Additi nal production of factor Xa can only occur through an alternative route involving factor IXa and the cofactor, factor VIIIa. This factor IXa results from the activation of factor IX by FVIIa/TF or by factor XIa resulting from factor XI's activation by thrombin.

Whether or n t this hypothesis will be substantiated in the future is unknown, but at present, many unresolved issues, together with conflicting experimental data that involve key areas of the pathway, make drawing a conclusion difficult. The technical problems that surround the reproduction of *in vivo* conditions, in experimental design, may account for the different observations made about the physiological significance of factor VII's enzymatic activity and also the mechanisms for factor XI activation. There are, however, other areas that have yet to be fully addressed.

The activation of factor X by factor IXa is considered essential in the revised pathway, but how is this factor IXa formed? If factor Xa activation by FVIIa/TF is inhibited by TFPI in complex with factor Xa, then so must the activation of factor IX by FVIIa/TF. If significant activation of factor IX therefore depends on factor XI, why isn't the bleeding experienced by factor XI deficient patients as severe as seen in patients with haemophilia? The procoagulant activity of factor XI present in platelets may provide the answer (80,81).

Factor VIIa has been used successfully in the treatment of haemophilia (82,83). This contradicts the reported neccessity of factor X activation by the tenase complex. TFPI, however, is unable to inhibit the action of FVIIa in complex with phospholipid (84). The suggestion is made that FVIIa/phospholipid complexes form and slowly activate significant amounts of factor X to compensate for the lack of tenase. Such a reaction would be ineffective at physiological concentrations of factor VIIa.

Conclusion

The tissue factor pathway of coagulation provides a revised formulation of blood coagulation. It combines the intrinsic and extrinsic pathways into one pathway which doesn't end with the initial production of thrombin and fibrin, but continues through feedback reactions. Although major advances have been made in understanding the mechanisms involved in coagulation since the waterfall/cascade pathway was first published, many questions still need to be answered before the tissue factor pathway of coagulation can be accepted as fact.

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